(a) providing a vector capable, in a transformant host cell, of expressing both a recombinant DNA sequence which encodes an active GS enzyme and the recombinant DNA sequence which encodes the complete amino acid sequence of the desired protein other than GS;

(b) providing a eukaryotic host cell which is a GS prototroph;

(c) transforming said host cell with said vector; and

(d) culturing said host cell under conditions which allow transformants containing an amplified number of copies of the vector-derived GS-encoding recombinant DNA sequence to be selected, wherein said transformants also contain an amplified number of copies of the desired protein-encoding DNA sequence.

76. The method of claim 75, wherein step (d) comprises culturing the transformed host cell in media containing a GS inhibitor and selecting for transformant cells which are resistant to progressively increased level of the GS inhibitor.

77. The method of claim 76, wherein the GS-encoding

C2 Contd

recombinant DNA sequence is under the control of a regulatable promoter.

6 ~~78~~. The method of claim 5 ~~77~~, wherein the regulatable promoter is selected from the group consisting of a heat shock promoter and a metallothionein promoter.

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7 ~~79~~. The method of claim 5 ~~77~~ or claim 6 ~~78~~, wherein the regulatable promoter is up-regulated during the culturing and selecting steps and is down-regulated after selection.

3 ~~80~~. The method of claim 2 ~~76~~, wherein the GS inhibitor is selected from the group consisting of phosphinothricin and methionine sulphoximine.

4 ~~81~~. The method of claim 2 ~~76~~ or claim 3 ~~80~~, wherein the media containing the GS inhibitor also contain methionine, whereby the concentrations of GS inhibitor in the media can be reduced.

8 ~~82~~. The method of claim 1 ~~75~~, wherein the desired protein is tissue plasminogen activator.

Subt C3

83. A method for co-amplifying a recombinant DNA sequence which encodes the complete amino acid sequence of a desired protein other than a GS, comprising:

(a) providing a first vector capable, in a transformant host cell, of expressing a recombinant DNA sequence which encodes an active GS enzyme;

(b) providing a second vector capable, in a transformant host cell, of expressing the recombinant DNA sequence which encodes the complete amino acid sequence of the desired protein other than GS;

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(c) providing a eukaryotic host cell which is a CS prototroph;

(d) transforming said host cell with both said first and said second vectors; and

(e) culturing said host call under conditions which allow transformants containing an amplified number of copies of the vector-derived GS-encoding recombinant DNA sequence to be selected, wherein said transformants also contain an amplified number of copies of the desired protein-encoding DNA sequence.

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84. The method of claim ~~83~~, wherein step (e) comprises culturing the transformed host cell in media containing a GS

inhibitor and selecting for transformant cells which are resistant to progressively increased of the GS inhibitor.

85. The method of claim ~~84~~, wherein the GS-encoding recombinant DNA sequence is under the control of a regulatable promoter.

~~86~~ 12. The method of claim ~~85~~ 11, wherein the regulatable promoter is selected from the group consisting of a heat shock promoter and a metallothionein promoter.

~~87~~ 13. The method of claim ~~85~~ 11 or claim ~~86~~ 12, Wherein the regulatable promoter is up-regulated during the culturing and selecting steps and is down-regulated after selection.

~~88~~ 14. The method of claim ~~84~~ 10, wherein the GS inhibitor is selected from the group consisting of phosphinothricin and methionine sulphoximine.

~~89~~ 15. The method of claim ~~84~~ 10 or claim ~~88~~ 14, wherein the media containing the GS inhibitor also contain methionine, whereby the concentrations of GS inhibitor in the media can be reduced.

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~~90~~. The method of claim ~~83~~, wherein the desired protein is tissue plasminogen activator.

Subtle 5 > 91. The method of ~~claim~~ 75 or claim 83, wherein the host ~~call is~~ a mammalian call.

18 1 9
~~92~~. The method of claim ~~75~~ or claim ~~83~~, wherein the host cell is a CHO-K1 cell.

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Subtle 6 > 93. A method for using a vector as a dominant selectable marker in a cotransformation process comprising:

(a) providing a vector capable, in a transformant host cell, of expressing a recombinant DNA sequence which encodes an active GS enzyme and a recombinant DNA sequence which encodes the complete amino acid sequence of a desired protein other than GS;

(b) providing a eukaryotic host call which is a GS prototroph;

(c) transforming the host call with the vector; and

(d) selecting transformant cells which are resistant to GS inhibitors, whereby transformant calls are selected in which the vector-derived GS-encoding sequence serves as a dominant selectable and co-amplifiable marker.

94. A method for using a vector as a dominant selectable marker in a cotransformation process comprising:

(a) providing a vector capable, in a transformant host cell, of expressing a recombinant DNA sequence which encodes an active GS enzyme;

(b) providing a second vector capable, in a transformant host cell, of expressing a recombinant DNA sequence which encodes the complete amino acid sequence of a desired protein other than GS;

(c) providing a eukaryotic host call which is a GS prototroph;

(d) transforming said host cell with both said first and second vectors; and

(e) selecting transformant cells which are resistant to GS inhibitors, whereby transformant cells are selected in which the vector-derived GS-encoding sequence serves as a dominant selectable and co-amplifiable marker.

95. A recombinant DNA vector comprising:

(a) a recombinant DNA sequence which encodes the complete amino acid sequence of a GS; and

(b) a recombinant DNA sequence which encodes the complete amino acid sequence of a desired protein other than said GS, the vector being capable, in a transformant host

cell, of expressing ~~both said~~ recombinant DNA sequences (a) ~~and~~ (b).

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~~96~~. A plasmid including the GS minigene from plasmid pSVLGS.1.

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~~97~~. A plasmid including the SV40-GS transcription unit from plasmid pSVLGS.1. --

REMARKS

Pursuant to 37 CFR 1.111, reconsideration of the Official Action dated May 24th, 1989 is respectfully requested.

In order to reduce the number of issues in the present case, previous claims 39 to 52, 56 to 61, 71 and 74 have been deleted without prejudice to Applicants right to file a divisional application relating to these claims at a later date.

Previous claim 53 now appears as new claim 95.

Equivalent to previous claims 54 and 55 in method form appear as new claims 77, 78, 85 and 86.

Previously claim 62 has been rewritten as new claim 83, and previous claim 63 has been rewritten as new claim 75. Previous claims 64 to 69, 72 and 73 appear as new claims